CONJUGATION, DIVISION, AND ENCYSTMENT IN *PLEUROTRICHA LANCEOLATA*.[1]

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Introduction.

Evidence has been steadily accumulating of recent years that many closely related species differ from each other in number of chromosomes, the numbers often being multiples, or multiples of some common factor. Because of this fact and because the processes of conjugation have been thoroughly studied in only a very few hypotrichs, the present investigation was undertaken. *Oxytricha fallax* and *Pleurotricha lanceolata*, although placed in different genera, are morphologically quite similar, and it was thought that a comparison of the two species with respect to chromosome number and details of conjugation would be of interest. It has been shown before in at least one case (*Chilodon uncinatus*, MacDougall, 1925) that new species may arise de novo from old ones among the protozoa, the chief differences being in chromosome number, and it is not unlikely therefore that this often happens in nature. A study of division and encystment was undertaken as a natural corollary.

Pleurotricha lanceolata and Similar Species.

Pleurotricha lanceolata was first described accurately by Stein in 1859, although two other species which he regards as the same had been described previously—*Stylonychia lanceolata* and *Keratium calvitium*, the former by Ehrenberg in 1832 and the latter by Müller. But Ehrenberg's organism had 16 to 18 cirri on the dorsal surface as well as the full complement on the

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ventral surface, and Müller failed to see the abdominal cirri, so that priority of description and name belongs to Stein.

Pleurotricha lanceolata was also described and figured by Kent (1880 to 1882), but his description is slightly different from that of Stein. In addition to the double row of marginal cirri on the left-hand side, Stein shows a very short third row in the middle region of the body which Kent omits, as well as the anterior third of the inner marginal row.

The race which I have used (Fig. 1) fits Kent's description very closely, but is only about half as large, and the inner marginal row of cirri is found only in the middle third of the body. Stein gives the size as varying between 83 and 143 μ, and Kent makes it just about twice as great. I have found the average size to be about 140 μ, the extremes being 100 to 165 μ, if exconjugants which are always exceptionally small are omitted.

Oxytricha bifaria (Stokes, 1887) also resembles *Pleurotricha lanceolata*, differing principally in having only a single marginal row of cirri extending around the body, and in having eight instead of six cirri on the anterior quarter.

There is also a considerable resemblance between *Oxytricha fallax* and the chief species under discussion. The former has five anal cirri arranged in an oblique row, the two posterior extending only slightly beyond the body margin.

Material and Methods.

The cultures were started from a single individual obtained from a mass culture of leaves and swamp water, which in turn was secured from Orient Springs, near Amherst, Massachusetts, November 5, 1925. These cultures were continued until May 1927, the medium being hay infusion, to which in some cases a small amount of peptone was added.

All preparations were fixed with Calkins' modification of Schaudinn's fluid, and stained with hemotoxylin, both the long and short methods being used.

Division.

The first indication of division is seen in the macronuclei, in each of which a nuclear cleft, or kernspalt, appears at the extreme

outer ends, and this gradually moves across the nuclei to finally disappear at the inner ends. Simultaneously, or very shortly afterward, a new adoral zone begins to form in the midregion of the body. This stage of division is shown in Fig. 2.

Very soon the micronuclei begin to enlarge and a clear space, or halo, appears about each. At about the same time a division center appears and divides, the two products remaining connected by a centrodesmose (Figs. 3 and 4). I have never been able to detect this endosome in the resting micronucleus however, and it is difficult to observe even in division. As the dividing nucleus enlarges it elongates, and the chromatin appears to be arranged in very fine strands, some of which seem to be in reality rows of granules (Fig. 4 *A*). At this time it stains very faintly. The chromatin next appears to become more or less concentrated at the poles (Fig. 4 *B*). As all this goes on the macronuclei change their shape, becoming almost round and the kernspalt finally passes off the inner ends (Figs. 4, 5, 6). The new adoral zone meanwhile increases in size, and new cirri may be seen developing beside the old ones of the old adoral zone (Fig. 6). In Fig. 5 the new ventral cirri may also be seen close to the adoral zones, where they first arise. As the time approaches for final separation of the daughter individuals these cirri gradually move to more nearly their normal positions (Fig. 11).

In the meantime the chromatin of the micronuclei appears to condense into discrete masses, which take the form of threads of varying length (Fig. 5 *B*). These gradually combine, until the nucleus appears to be composed of closely packed and more or less tangled threads, as in Figs. 5 *A* and 6 *B*, and finally a definite spindle appears (Fig. 6 *A*). The poles of the latter move apart and a very characteristic figure is produced which is shown in Fig. 7 *C*. Here the chromosomes appear as heavily staining Vs, the limbs of which are connected by heavy and deeply staining strands. Apparently division is really longitudinal, the two daughter chromosomes taking on a V shape and appearing connected at the ends for a while thereafter. Their final separation is shown in Fig. 7 *A*. Fig. 8 *A* shows a somewhat later stage of the metaphase.

At about this time the macronuclei begin to elongate (Fig. 8) and the remaining stages in division are passed through very rapidly, the time required being about fifteen minutes at ordinary temperatures, as compared to more than an hour for the preceding steps. The micronuclei enter the anaphase, which is quickly completed, requiring only a very few minutes, and which may be seen in Figs. 9, 9 *A*, 9 *B*, 9 *C*. The telophase is shown in Figs. 10, 11, 11 *A*, 11 *B*, and 12. The macronuclei divide at this time (Figs. 11 and 12), and the parent animal very shortly separates into two daughter individuals (Fig. 13).

Discussion.

The process of division in ciliates is so well-known that few comments are necessary, since different species differ only in minor details, and fission in *Pleurotricha lanceolata* follows the general plan. There are, however, a few points in which this species differs from others, and in other respects there are interesting resemblances.

The presence of a kernspalt is said to be common to all hypotrichous ciliates, even during the major part of the resting stage (Calkins, 1919), but I have never been able to see it in *Pleurotricha* except when the animal was about to divide, as shown by the presence of a rudimentary adoral zone. This happens even before there is any visible change in the micronuclei.

Apparently the early appearance of a new cytostome with its accompanying peristomial apparatus is characteristic of most dividing infusoria, *e.g. Uroleptus mobilis* (Calkins, 1919), *Chilodon uncinatus* (MacDougall, 1925), although there are other cases in which its development is more delayed as in *Paramœcium trichium* (Wenrich, 1926).

It seems to be generally accepted that in division there is a complete reorganization of the entire cell, extending even to the disappearance and subsequent reformation of all the organelles, including trichites in organisms which have them, undulating membranes, cirri, and even cilia. According to Wallengren (1900) the cirri of the old adoral zone in hypotrichous ciliates are gradually absorbed as new ones take their places, and I have

been able to confirm this observation for *Pleurotricha*. That this reorganization does not always extend to the old oral apparatus, however, is indicated by Wenrich (1926) who was unable to find evidence that the old cytostome disappeared and was reformed in *Paramœcium trichium*.

It is a remarkable fact that all the cirri, with the exception of the marginal ones, arise in the immediate neighborhood of the adoral zones, and indeed the first indications of their appearance are visible very shortly after the adoral zone itself appears. That this was the case in the Oxytrichidæ was noticed by Sterki (1878), and has been noted for other groups by other investigators. MacDougall (1925) found that new cilia in a dividing *Chilodon uncinatus* first appear near the new pharyngeal baskets.

The presence of a centrosome which divides as an initial step in division has until recently not been noted in ciliates. It was reported by Calkins in the first maturation division of *Uroleptus mobilis* however (Calkins, 1919), although he evidently did not see it in ordinary division, and MacDougall found it in *Chilodon uncinatus*. To this list can now be added *Paramœcium trichium* (Wenrich, 1926), and *Pleurotricha lanceolata*.

Encystment.

Encystment is a common occurrence in the life cycle of *Pleurotricha bifaria*, although what the conditions are which determine its occurrence I have been unable to discover. It may occur in old cultures, and is perhaps most frequent under those circumstances, but it also occurs in pedigreed drop cultures, which are changed every day, and in which the division rate is rapid. Of four individuals which have arisen by division from a single ancestor during the preceding twenty-four hours it is not uncommon to find one or two encysted, while the others continue to divide actively.

The account of nuclear phenomena which follows is admittedly inadequate, but it is given since no other description has been made of this stage in the life cycle of this particular species, and because the history of the cytological changes which occur in ciliates during encystment is notoriously incomplete.

The first change appearing in an individual about to encyst is

like that occurring in other infusoria under similar circumstances. The shape becomes spherical, the contractile vacuole gradually ceases to pulsate, the food vacuoles and all the organelles disappear (Fig. 14). The next step is the secretion of the cyst wall, shown in Fig. 15. It is thick, quite pliable but very tough, and without visible openings. The outside is covered with irregular, short spines. Such a cyst is shown by Stein (1859) for *Pleurotricha lanceolata*, but Cienkowsky (1855), who gives three figures of the cyst formation of *Stylonychia lanceolata*, does not show the spines. Nevertheless these two species are regarded by Stein as the same, as already stated.

Soon (probably under normal conditions in about twenty-four hours) the old macronuclei, and apparently one of the micronuclei, are bodily extruded through the cyst wall, although the opening through which they pass is never visible under any other circumstances (Fig. 16). This leaves the encysted animal in the condition shown in Fig. 17. The remaining nucleus then proceeds to divide but I am unable to say much as to the details of this division. Apparently some of the micronuclei shown in Figs. 18 and 19 are in process of division, and the process appears to be a typical mitosis. As to how a cyst in the condition of that in Fig. 19, just referred to, reaches that shown in Fig. 20 I am unable to say. Cysts are exceedingly difficult to stain and destain at this stage.

Figs. 20 and 21 show the final stages in the reorganization. As may be seen there has been a great decrease in the quantity of cytoplasm, and a corresponding decrease in chromatin. Animals at this stage may be seen swimming about within the cyst, but I have never been able to get them to leave the cyst, despite washing, prolonged observation in cultures in which the media was changed daily, and attempts to rupture the cyst wall artificially. The latter always failed because of the tough pliable nature of the wall. Both distilled water, tap water and Ringer's solution were used for washing.

Moore (1924) found that *Spathidium spathula* behaved in a similar fashion, though she was more successful in liberating encysted individuals by puncturing the cyst wall. Under ordinary circumstances few encysted animals would excyst, and

drying, which Calkins (1919) found necessary to induce the excystment of *Uroleptus*, and the addition of fresh medium, found efficacious by Moody (1912) in securing the excystation of *Spathidium*, both proved unsuccessful.

But the cytological changes which occur during the encystment of *Spathidium spathula* appear to be markedly different from those which take place in *Pleurotricha*. There is no extrusion of nuclear or cytoplasmic material from the cyst in Spathidium, but the macronuclei degenerate and finally disappear, the fragments being absorbed by the cytoplasm. The micronuclei remain practically unchanged. Shortly before redifferentiation occurs, preparatory to leaving the cyst, new macronuclear anlagen appear which strongly resemble those formed in the late stages of conjugation. It is possible that more study would show that the new macronuclei arise in a similar way in *Pleurotricha*, but I have seen no indication of it in any of my preparations.

That an extensive nuclear reorganization takes place in many infusoria during encystment has been known for a long time, but I have found no mention of the extrusion of nuclear material in any other species, although Prowazek (1899) believed that such a phenomenon occurred during conjugation. Fermor (1913) found that in *Stylonychia* there was a fusion of the two micronuclei previous to the formation of a new nuclear apparatus, and it is possible that the same thing occurs in encysted individuals of *Pleurotricha*. But in one instance I have actually observed one of the two micronuclei being extruded along with the macronuclei.

Conjugation.

During the eighteen months in which the cultures were maintained conjugation occurred but rarely, and epidemics of it were never observed. Even in mass cultures containing thousands of individuals it was usually necessary to look for an hour or more to obtain a few pairs. Because of the small number of conjugants no sections were made and all the results herein described were obtained from whole mounts, fixed with Calkins' modification of Schaudinn's fluid, stained with hematoxylin, and mounted individually. Various methods of inducing conjugation have been

suggested by investigators who have found them more or less useful with certain species, and a number of these methods were tried in this case, but with practically no success.

Conjugating individuals fuse by the adoral surfaces (Fig. 22), the entire peristome of one member of the pair disappearing completely. There seems to be no reason why it cannot function in the other conjugant but there is very little evidence that it does so, for food vacuoles become fewer in number as conjugation progresses, and exconjugants are always small. The time from fusion to final separation varies from eighteen hours to five days, but the usual duration is twenty-four hours.

Behavior of the Micronuclei.

The micronuclei normally go through three maturation divisions, only two taking part in each. The others degenerate more or less rapidly, although some may persist even after the interchange. The pronucleus usually undergoes two cleavage divisions. Of the four products one enlarges and eventually gives rise to the macronuclei of the reorganized exconjugant, one degenerates, and the remaining two form micronuclei.

The First Maturation Division.

This division requires more time than any of the others—at least eight hours—and is also strikingly different in type. The micronuclei at first show no change but soon increase in size, become surrounded by a clear space, or "halo," and stain more faintly than usual. The chromatin takes on a finely-granular appearance. Shortly an endosome, or division center, appears, and in favorable preparations two may be seen (Fig. 28 *A*, *C*), but I have never been able to detect an intradesmose connecting them, although in vegetative division and in the other divisions of conjugation it can often be seen. This division center increases in size until it becomes hemispherical and stains very heavily (Fig. 28 *C*, *D*). In the meantime spindle fibers begin to appear, and the spindle takes on the typical parachute appearance (Fig. 28 *C*, *D*, *E*). The chromatin in the expanded top of the parachute, which is at first composed of very small, dimly staining granules, condenses into larger granules which stain

more heavily and these pair to form heavily staining chromosomes of dumbbell shape. These granules are altogether too numerous to count, and in most preparations this is also true of the chromosomes, but I have made counts in a few cases which will be discussed at greater length below. The chromosomes now move to the center of the spindle and divide longitudinally (Fig. 28 *F*, *G*). Stages of the anaphase and telophase are shown in Figs. 28 *H* to *M*. The most characteristic feature of this division, aside from the dumbbell shape of the chromosomes, is the way in which they lag and the peculiar curve at the poles of the spindle.

The Second Maturation Division.

This follows rapidly on the first, and is over in much less time, with the incidental result that material showing it is difficult to obtain. It is also of very different character. The micronuclei which are to divide enter the prophase almost before the telophase of the preceding division is complete (Fig. 32 *A*). At this stage they stain faintly, and rows of granules appear which are arranged in a more or less "whorled" fashion (Fig. 32 *B*). Fig. 32 *C* represents a stage which is seldom seen, but it is presumably earlier than those shown in the two preceding figures. The nucleus next enlarges and the rows of granules become threads (Fig. 32 *D*). This may well be in reality a leptotene stage, since reduction occurs during this division. There is soon evidence of a definite spindle which is at first of a peculiar oval shape (Fig. 32 *E*, *F*). I have been unable to get preparations showing the metaphase and later stages of the anaphase, but the early anaphase is shown in Fig. 32 *F*. I have made a number of counts of the number of chromosomes concerned in this division, the average result being forty. Two stages of the telophase are shown in Fig. 32 *G* and *H*.

The Third Maturation Division.

The third division differs in type from both the preceding, and requires more time than the one just described. The micronuclei which are to divide enlarge, and become very finely granular. I am uncertain as to whether there is an intradesmose

connecting the products of the division of the intranuclear endosome, but I believe that there is. What is apparently one end of it can be seen in Fig. 36 *A*. These granules now condense to form rows, and the latter in turn become much coiled chromosomes which often appear double (Fig. 36 *B*, *C*). The metaphase is shown in Fig. 36 *D*, and is considerably like that of ordinary vegetative division. The chromosomes in both the second and third maturation divisions are rod-shaped, in contrast to the dumbbell shape which they have in the first, and the V and irregular rod shape of the cleavage divisions. The long-pointed anaphases are characteristic of the third division, and long drawn-out telophase (Figs. 36 *E* to *I*).

The interchange is a rapid process, and the wandering nuclei do not appear to differ in any respect from the stationary ones. New adoral zones appear at this time or very early during the first cleavage division (Fig. 37).

The First Cleavage Division.

The amphinucleus is shown in Fig. 40, and is apparently divided into two parts, doubtless representing the two pronuclei. The stages in the first cleavage division are shown in Figs. 41 *A* to *H*. The nucleus, which increases very much in size shortly after fusion, becomes smaller, and forms a peculiar sort of spindle (Fig. 41 *A*), in which the chromatin appears to be condensed into a heavy ribbon, twisted more or less upon itself, and quite definitely double. Chromosomes soon appear which are arranged in bouquet fashion, about a small endosome, and which at this stage seem to be, in some cases at least, in pairs (Fig. 41 *B*). They then straighten out and form a characteristic spindle which has at first two definite parts, possibly representing the two pronuclei (Fig. 41 *C*). At this time the chromosomes are definitely double, and apparently twisted about each other. The metaphase is figured in 41 *D*, and the anaphase soon follows. Apparently the chromosomes in the later stages of this division are shaped like very acute Vs. Figs. 41 *E* to *H* show the anaphase and telophase. The former is rather characteristic in its early stage because the chromosomes are so widely separated within the receding plates.

The Second Cleavage Division.

The first cleavage division which has just been described is a rapid process, but the second one is much slower. A division center appears, as in two of the three maturation divisions, and divides, the products remaining connected by an intradesmose (Fig. 46 *A*). The nucleus then becomes elliptical, and the chromatin becomes arranged in long deeply-staining strands which are at first quite regular in arrangement (Fig. 46 *B* and *C*). These elongate and become twisted (Fig. 46 *D*, *E*), some of them appearing double. The whole spindle resembles nothing so much as a tangled skein of yarn at this stage, and possibly the stage represented in Fig. 46 *D* is in reality a spireme. The strands in the following figure are definitely polarized and probably are really chromosomes. The metaphase is shown in Fig. 46 *H*. It does not differ very much from that in the first cleavage division. The anaphase is very peculiar, as can be seen from Figs. 46 *J* to *M*. At the poles of the spindle a "cap" of very deeply-staining chromatin is formed and the chromosomes lose the definite shape which they had in the metaphase, apparently coalescing into more or less irregular masses which take a very heavy stain. These are very characteristic and make the later stages of the second cleavage division differ from all the others. The strands persist for some time in the anaphase (Fig. 46 *L*, *M*).

The Occasional Third Cleavage Division.

Following the second division there may in some cases be another, but when it occurs only two of the four nuclei take part as a rule, so that exconjugants in which this third division has taken place have six nuclei, rarely eight. All the stages of this division which I have observed are like corresponding ones of the second division.

The Behavior of the Macronuclei.

The anterior of the two macronuclei elongates during the maturation divisions, and may occasionally divide by a process of mass division, but only in very rare cases. At the same time it undergoes a slow fragmentation, so that the cytoplasm usually

has a number of heavily staining masses of chromatin at this time, which often simulate degenerating micronuclei derived from previous divisions. The posterior macronucleus may elongate slightly, but I have never observed it to divide and it undergoes much less fragmentation than the other.

The chromatin fragments and degenerating micronuclei usually disappear about the time of the interchange, or soon afterward, but may in some cases persist until after the cleavage divisions.

Reorganization of the Exconjugant.

Reorganization begins before separation, and usually requires several days. One of the four nuclei resulting from the second cleavage division becomes very large and coarsely granular, and at the same time loses much of its capacity to stain with hemotoxylin. It is destined to form the macronuclei of the reorganized exconjugant and is shown in Figs. 47, 48, 49, 50, 52 and 53. This nucleus divides at the first division of the exconjugant, and then divides again without corresponding division of its possessor, thus restoring the normal macronuclear condition (Fig. 55).

But it is apparently possible for reorganization to become complete without prior division of the exconjugant, and an individual in which this has happened is shown in Fig. 54. It may be that this can happen in individuals in which there has been a third cleavage division.

The micronuclei of the reorganized conjugant are derived directly from two of the three which remain from the second cleavage division. The third persists for a time but eventually degenerates. As to what happens when there have been three cleavage divisions I am unable to say, since I have been unable to observe enough cases.

The remains of the old macronuclei persist for a day or two as more or less circular, deeply staining and vesicular masses of chromatin (Figs. 49 to 52), but they eventually disappear completely, leaving no trace (Fig. 53).

The Number of Chromosomes.

In order to determine the number of chromosomes a large number of counts were made in several different stages, par-

ticularly from an especially favorable preparation showing the early anaphase of the first maturation division, and from several preparations of the anaphase of the third maturation division.

Since the total number of chromosomes is so large in the anaphase of the first maturation division accurate counts are difficult, but ten were made as a check for the other counts described below. The mean of these ten counts was eighty-six, which would indicate that the diploid number is forty-three. The standard deviation was found to be 15.4, the coefficient of variation 17.9, and the probable error $\pm$ 3.46. There is no doubt therefore that the counts are very significant.

A few counts were made of the chromosomes in the early anaphase of the second maturation division, and the average was close to forty, again indicating therefore that this figure is close to the diploid number.

Since the most favorable preparations for chromosome counting were those of the third maturation division these were given the most study. To insure results as free from error as possible seventy counts were made from several preparations showing the anaphase of this division. A curve was constructed of these counts and the mode found to be 19. The mean was 19.62, and the probable error $\pm$ .295. The coefficient of variation was 18.6 per cent., and the standard deviation 3.66. It is therefore certain that the counts are highly significant, and there is no doubt that the haploid number of chromosomes is close to twenty, with a high degree of probability that it is exactly that number, making the diploid number forty.

Since the number of chromosomes in *Oxytricha fallax* is twenty-four (Gregory, 1923), there is therefore no obvious relation as far as number of chromosomes is concerned between this species and *Pleurotricha lanceolata*.

Discussion.

Although only a relatively small number of ciliates have been studied with reference to the phenomena of conjugation the process appears to be essentially the same in all. From his own observations Maupas (1888) divided it into eight phases—*A*, the period of preparation preceding the first meiotic division; *B*,

the first division; *C*, the second division; *D*, the third division; *E*, the interchange and fusion of the pronuclei; *F*, the first cleavage division; *G*, the second cleavage division; and *H*, the period of reorganization preceding the first fission of the exconjugant.

These phases have been shown to hold for all infusoria so far studied, including *Pleurotricha lanceolata* which is the subject of this paper, although in a few forms such as the Vorticellidæ, Ophryoscolecidæ, and *Euplotes patella*, there are one or more preliminary divisions before the meiotic divisions begin. (In the case of the Vorticellidæ this happens only in the case of the microgamete).

Ciliates as far as at present known fall into two classes according to the behavior of the micronuclei in the first phase—those which undergo a prophase like *Paramœcium*, the micronucleus being drawn out into a crescent, and those in which a parachute or candelabra-like figure is formed, to use Calkins' term. To the last group belong *Onychodromus grandis* (Maupas, 1888), *Bursaria truncatella* (Prowazek, 1899), *Didinium nasutum* (Prandtl, 1906), *Anoplophrya branchiarum* (Collin, 1909), *Uroleptus mobilis* (Calkins, 1919), *Oxytricha fallax* (Gregory, 1923) and *Chilodon uncinatus* (MacDougall, 1925). The character of the first division, already described, makes it necessary to add *Pleurotricha lanceolata* to this list.

As might perhaps be expected from the close morphological resemblance of *Oxytricha fallax* and *Pleurotricha lanceolata* the details of the conjugation process are much alike, and while they are also similar to those which occur in *Uroleptus mobilis* as described by Calkins, the resemblance is much less striking.

The formation of the parachute preliminary to the first maturation division more nearly resembles the corresponding stages in *Uroleptus* than in *Oxytricha*, but differs from both. In the latter, although the parachute fibers are focused on a single granule derived by division from the endosome just as they are in *Pleurotricha*, there is also a centrodesmose connecting the two halves of the endosome which I have never been able to observe in *Pleurotricha*. In *Oxytricha* there appears to be no endosome, and the place of the basal granule is taken by a row of granules.

The chromosomes in this division appear to be formed by the fusion of the granules into which the chromatin is divided early in the prophase, but whether the number of these granules bears any constant relation to the number of chromosomes, as Gregory believes it does in the case of *Oxytricha*, I am unable to determine. These chromosomes which at first make up the top of the parachute appear to move down the fibers until they reach the center of the spindle when they form an equatorial plate. The other pole of the spindle appears to be formed by one of the two halves of the endosome which remains in the top of the parachute, the other as already stated, forming the granule at its base. This is apparently the same as in *Uroleptus*.

The number of nuclei taking part in any of the three maturation divisions is apparently never greater than two in the case of *Pleurotricha*, although forms in which there are three micronuclei occur. Of the four products of the first division all but two degenerate, and the same thing happens after each of the other two maturation divisions. The number of nuclei taking part in each maturation division in *Oxytricha* appears to be variable, although there are always at least two. In other multinucleate ciliates which have been studied there is considerable variation in this respect. In *Bursaria* all the sixteen or more nuclei may take part in the first meiotic division (Prowazek, 1899), and in *Uroleptus* there may be two, three or four primary spindles (Calkins, 1919), but these are exceptions.

The second maturation division is apparently of different character from the first in most ciliates, and lacks the elaborate preliminaries of the latter, with the result that it requires much less time. The micronuclei in most cases do not return to the resting stage between the first and second division, although there are exceptions (*e.g.*, *Chilodon*, MacDougall, 1925). In all these respects therefore *Pleurotricha lanceolata* is typical.

In nearly all cases in which a careful study has been made reduction has been found to take place in the second meiotic division, although the first division was thought to be the reducing division in *Paramœcium caudatum* (Calkins and Cull, 1907), and in *Oxytricha fallax* (Gregory, 1923). But Dehorne (1920) regards the third division as the one in which reduction occurs in the

former form. In the case of *Pleurotricha* there is no doubt but that it occurs in the second, for the following reasons: The total number of chromosomes taking part in the first division in the beginning anaphase has been determined to be in the neighborhood of eighty, as already stated, whereas there are only half as many concerned in the second division, and the average number moving toward each pole of the third maturation spindle has been determined, as stated above, to be about twenty.

The only protozoa in which reduction is known to occur at any other time (with the two exceptions already mentioned), are *Diplocystis* (Jamieson, 1920), and *Aggregata eberthi* (Dobell, 1915). It is suggested by Dobell and Jamieson that this may be a universal occurrence among the Telosporidia.

The third maturation division and the interchange take place exactly as in *Oxytricha*, and are very much alike, even to details in the form of the spindle. Nothing is said as to the existence of a division center or intradesmose however. The dumbbell character of the chromosomes is not lost until the third division in the case of *Oxytricha*, and it is stated that the chromatin streams toward the poles in the form of granules. I find that the chromosomes are definitely rod-shaped in *Pleurotricha*, in both the second and third divisions, although the rods are heavier and shorter in the former, and may give place to rows of granules in the late anaphase stages of the latter. The dumbbell character is never regained after the first of the three maturation divisions.

The two cleavage divisions follow the usual course, again resembling closely those in *Oxytricha*, although not many details are given in Gregory's paper. The products of the second cleavage division are equal as in *Oxytricha*, but differ in this respect from the state of things in *Uroleptus mobilis*, in which one of the two nuclei derived from the first division gives rise to the macronuclei and the other to the micronuclei of the reorganized exconjugant (Calkins, 1919). I have found no other case in which the anaphase of the second cleavage division resembled that in *Pleurotricha* however, and it differs strikingly from that in *Oxytricha*.

The occasional third cleavage division is also a striking charac-

teristic, and as far as I can discover it occurs in no other hypotrich so far described, although it is admitted that the number studied is thus far small. In other forms such as *Paramœcium caudatum* (Calkins and Cull, 1907), and several of the Vorticellidæ, a third division of the amphinucleus occurs, but as a regular thing. In *Bursaria* (Prowazek, 1899), there are four divisions before differentiation occurs.

The behavior of the macronuclei in *Pleurotricha* follows the usual rule—degeneration eventually occurs, although it is not completed until after separation. But division of the macronuclei, although apparently the rule in *Oxytricha*, is very rare in *Pleurotricha*.

The time required for reorganization and the details of the process differ considerably in different cases, but the process as it occurs in *Pleurotricha* is not significantly different from the essential features of reorganization in other species. Prowazek believed that the remnants of the old macronuclei must be extruded bodily from the cell in *Stylonychia pustulata* and *Bursaria truncatella* (1899), but I have seen no evidence of this in the present instance, nor has it ever been observed by anyone else. He based his hypothesis on the fact that nucleins are indigestible.

The Physiological Effect of Conjugation.

The significance of conjugation has been much disputed, one school holding that it is the necessary result of senescence in any given race, and that by it the death of the race could be averted and rejuvenation, expressed chiefly in an acceleration of the division rate, be secured. Maupas held this view and since his time Calkins has been its leading exponent. Another group has regarded conjugation as a process useful chiefly as a means of producing variations, which are much greater and much more frequent in exconjugants than in ordinary vegetatively reproducing individuals. Jennings has been one of the chief expounders of this view.

Many experiments have been tried to settle this question and since the results have differed considerably for different species I have endeavored to find out the effect of conjugation in *Pleuro-*

tricha lanceolata, as measured by the division rate of exconjugants when compared to closely related non-conjugating lines.

The experiment consisted in isolating one hundred exconjugants, and following them daily as long as possible. Of this number 92 per cent. died without division, usually about four days after separation, and of the remaining eight only one gave any evidence whatever of rejuvenation. The others divided two or three times and then all died. This single individual gave rise to a line which divided between two and three times a day for six days, and from then until the death of the line four weeks later, the average daily division rate gradually declined until during the last two weeks it was much less than one per day.

The controls during this period averaged from one to two divisions a day, and showed no evidence of senescence for the six months for which pedigreed cultures were maintained. Both controls and exconjugants were in the same media.

It is realized that one hundred cases is scarcely enough to draw final conclusions from, and yet the percentage of deaths is so high that it does not seem likely that a greater number of cases would have given significantly different results.

Jennings (1913) in a long and comprehensive series of experiments demonstrated that with *Paramœcium* "Conjugation decreases the rate of fission, causes a great increase in variation in fission rate, brings about many abnormalities, and greatly increases the death rate," but Mast (1917), using *Didinium nasutum*, was unable to secure evidence of any such effect. He found that there was no appreciable effect on either death rate, fission rate, or increase in variation of fission rate, thus proving however that there was also no rejuvenation.

Calkins, in a long series of experiments with *Uroleptus mobilis*, has shown that in this species at least conjugation appears to have a genuine rejuvenating effect (Calkins, 1919 and 1926), and Woodruff and Spencer (1924) conclude that conjugation has a similar effect in the case of *Spathidium spathula*, although they prefer the term "high survival value" to that of "rejuvenation."

It has been shown by many investigators working with various species that as culture methods are improved the longevity of cultures without conjugation can often be indefinitely increased,

so that it can probably be safely concluded that conjugation is at least not a necessary process if environmental conditions are sufficiently favorable.

Since cultures of *Pleurotricha* have been maintained for eighteen months with very little conjugation (none in the pedigreed lines which were carried for six months), and since in the cases mentioned above none of the exconjugants gave rise to lines which continued for any length of time, although kept under identically the same conditions as controls which maintained a uniform and fairly high division rate, it can be concluded I think that conjugation is not an indispensable part of the life cycle. This conclusion is supported by Baitsell (1914) who carried on experiments of this nature with both *Pleurotricha lanceolata* and *Oxytricha fallax*.

It is nevertheless apparent that a process which is so universal among infusoria as conjugation must serve some useful purpose, and that in nature exconjugants do not always die. Why then do they die in cultures? It is impossible to give a definite answer, but it has been suggested that media which is suitable for active vegetative multiplication may not always be suitable for conjugation and exconjugants, and this may well be the explanation.

Summary.

1. *Pleurotricha lanceolata* is a hypotrichous ciliate belonging to the family Pleurotrichidæ. The species has as its chief characteristic six anal cirri, divided into two groups. The anterior of the two groups consists of four cirri—three very large ones arranged in an oblique row, and a smaller one, a little forward of the posterior end of the row. The second group includes two cirri, both of them very long and projecting well beyond the posterior end of the animal. The usual size of vegetative individuals is 140 μ.

2. The process of division does not differ particularly from that in other infusoria, except that in the very early stages an endosome appears and divides, the products remaining connected by an intradesmose. This has been described in *Paramœcium trichium* but in few if any other species. The process of division is initiated by the appearance of a rudimentary adoral zone and

a kernspalt in each of the macronuclei. The micronuclei divide by typical mesomitosis. When division of the latter is virtually complete the macronuclei follow suit, dividing amitotically, and then the cell itself divides. All organelles appear to be regenerated, the old ones being absorbed.

3. Encystment may occur at any time, and appears to bear no relation to periods of depression, to division or to conjugation. The old macronuclei are extruded bodily from the cell, and a single micronucleus remains. It is uncertain whether the other is always extruded with the macronuclei or fuses with the first micronucleus. An uncertain number of micronuclei are formed from the single remaining micronucleus, and from these the normal nuclear complex is rebuilt, the process being complete at the time the animal is ready to leave the cyst.

4. The nuclear changes which occur during conjugation are essentially the same as those described for other ciliates. There are three maturation divisions, an interchange of pronuclei, and two or rarely three cleavage divisions. The four products of the second division are at first alike, but one soon enlarges and eventually gives rise to the new macronuclei of the reorganized exconjugant. One of the others degenerates and the other two form the new micronuclei. The old macronuclei degenerate after separation of the exconjugants. There may or may not be one division of the latter before reorganization is completed.

5. Reduction occurs in the second maturation division. The diploid number of chromosomes is forty, as nearly as can be determined, and the haploid number twenty. The chromosomes are dumbbell-shaped in the first maturation division, and rod-shaped in the second and third. They are also rod-shaped in the cleavage divisions, but the shape then is unlike that in the maturation divisions or in vegetative division.

6. Each of the divisions in conjugation differs from all the rest and from vegetative division.

7. Conjugation occurs but rarely in the race of *Pleurotricha* used, and under the conditions of culture virtually always results in the death of the conjugants a few days after separation. In only one instance out of a hundred did it result in anything like rejuvenation, and even in this case the daughter race died within a month.

8. Although the details of conjugation are much alike in *Oxytricha fallax* and *Pleurotricha lanceolata* there is no obvious relation between the number of chromosomes of these two very similar species.

Conclusion.

The cytology of conjugation and division in *Pleurotricha lanceolata* has been described in detail, together with some of the cytological phenomena of encystment. The processes of division do not differ particularly from those which are known to occur among ciliates in general, with the exception of the dividing endosome and connecting intradesmose. Certain features in the division of the micronucleus are strikingly like some which occur in the mitosis of metazoan tissue cells.

A remarkable feature of encystment is the extrusion of the old macronuclei, and perhaps one of the micronuclei, through the cyst wall. After this has happened the normal nuclear complex is rebuilt from the single micronucleus remaining. Whether the presence of the latter is regularly due to the extrusion of the other micronucleus from the cell, or to the fusion of the two original micronuclei is not altogether certain.

The cytological changes of conjugation are in general similar to those previously described in other ciliates, and especially resemble those which occur in *Oxytricha faliax*, but there are important differences in detail. Reduction is shown to occur in the second maturation division. The diploid number of chromosomes is too great to determine exactly, but is probably forty. There may be a third cleavage division in addition to the two which regularly occur, and in this respect *Pleurotricha lanceolata* differs from any other hypotrich so far described.

Conjugation appears to be not only an unnecessary part of the life cycle of this species, at least as long as environmental conditions remain favorable, but is a very dangerous event, for 92 per cent. of one hundred exconjugants died without further division, and only 1 per cent. showed any indication of an accelerated fission rate.

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EXPLANATION OF THE FIGURES.

Magnification (unless otherwise stated) × 550; spindles × 1700.

Plate I.

Early Stages of Division.

Fig. 1. A typical vegetative individual. Occasionally individuals are found with only one micronucleus, or with three.

Fig. 2. The first stage in division. A kernspalt appears at the outer end of each macronucleus, and the beginning of a new adoral zone can be made out (a. z.).

Fig. 3. The micronuclei show an endosome, or division center, in process of division, the halves being connected by an intradesmose. The kernspalt has moved nearer the center of each macronucleus

Fig. 4. The micronuclei have become somewhat larger, and the macronuclei have become almost round. The kernspalt is almost ready to pass off the latter entirely, and the new adoral zone is more conspicuous.

Fig. 4 *A*. An elongated, faintly staining micronucleus; seen in very early division.

Fig. 4 *B*. Same as above, but a little later.

Fig. 5. The chromatin in the micronuclei has condensed into definite threads. The macronuclei have become almost completely spherical and the new adoral zone is well developed. New cirri are making their appearance in the neighborhood of both adoral zones. Enlarged figures of the micronuclei are given under 5 *A* and *B*.

Fig. 6. The anterior micronucleus, shown enlarged in Fig. 6 *A*, has formed a definite spindle.

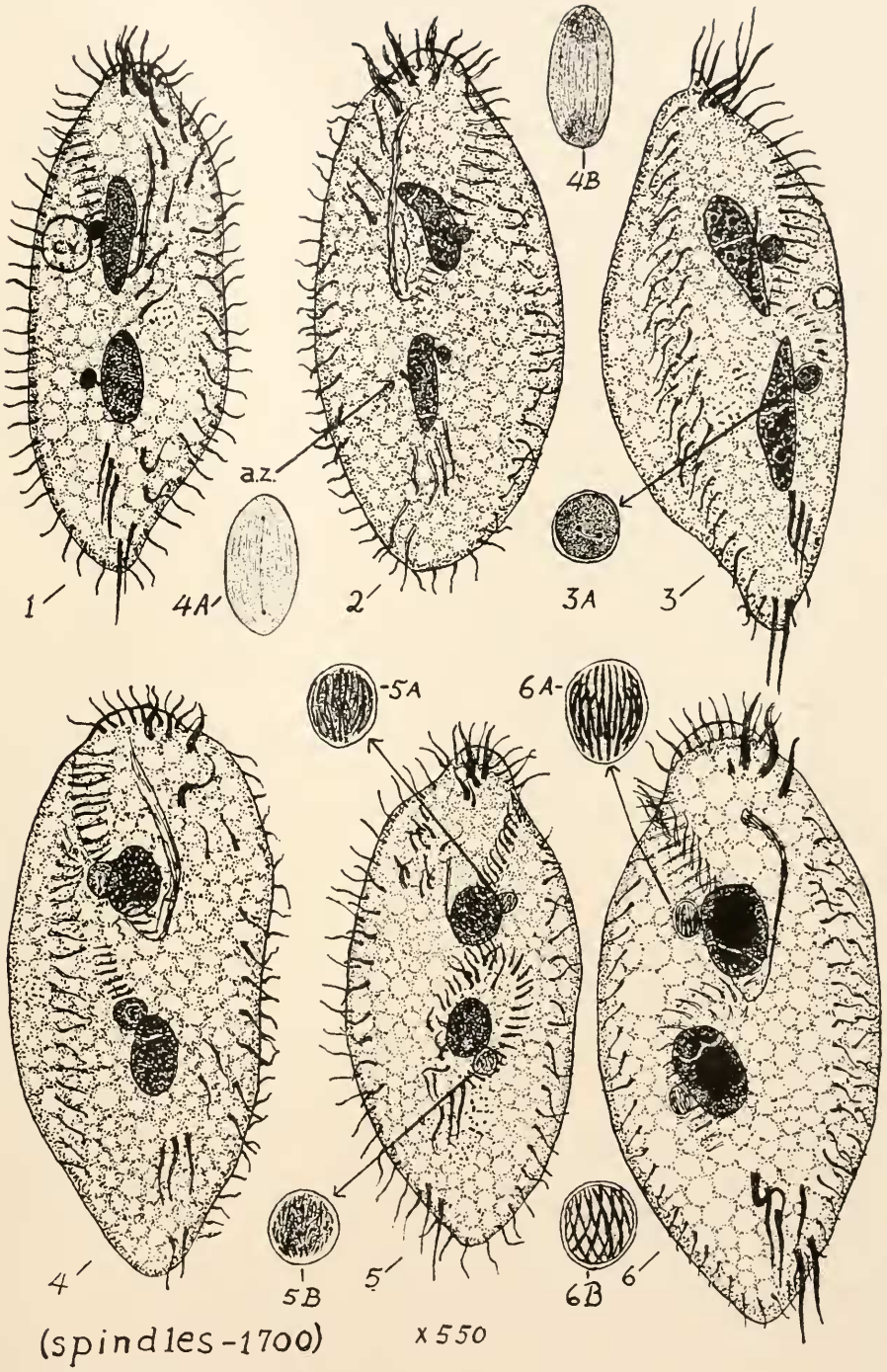

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Plate II.

Later Stages of Division.

Fig. 7. Both micronuclei have formed spindles, which are shown enlarged in 7 *A* and 7 *B*. The cirri are larger, and the macronuclei still almost round.

Fig. 7 *C*. A spindle, magnified about 3500 times, showing the shape of the chromosomes, and the way in which they divide. The heavy strands connecting them are to be noted particularly.

Fig. 8. An individual with but one micronucleus, which is in the metaphase. The macronucleus have elongated, and the new cirri are conspicuous and large.

Fig. 9. An individual in which the micronuclei are in the anaphase.

Fig. 9 *C*. A later stage in the anaphase.

Fig. 10. A still later anaphase.

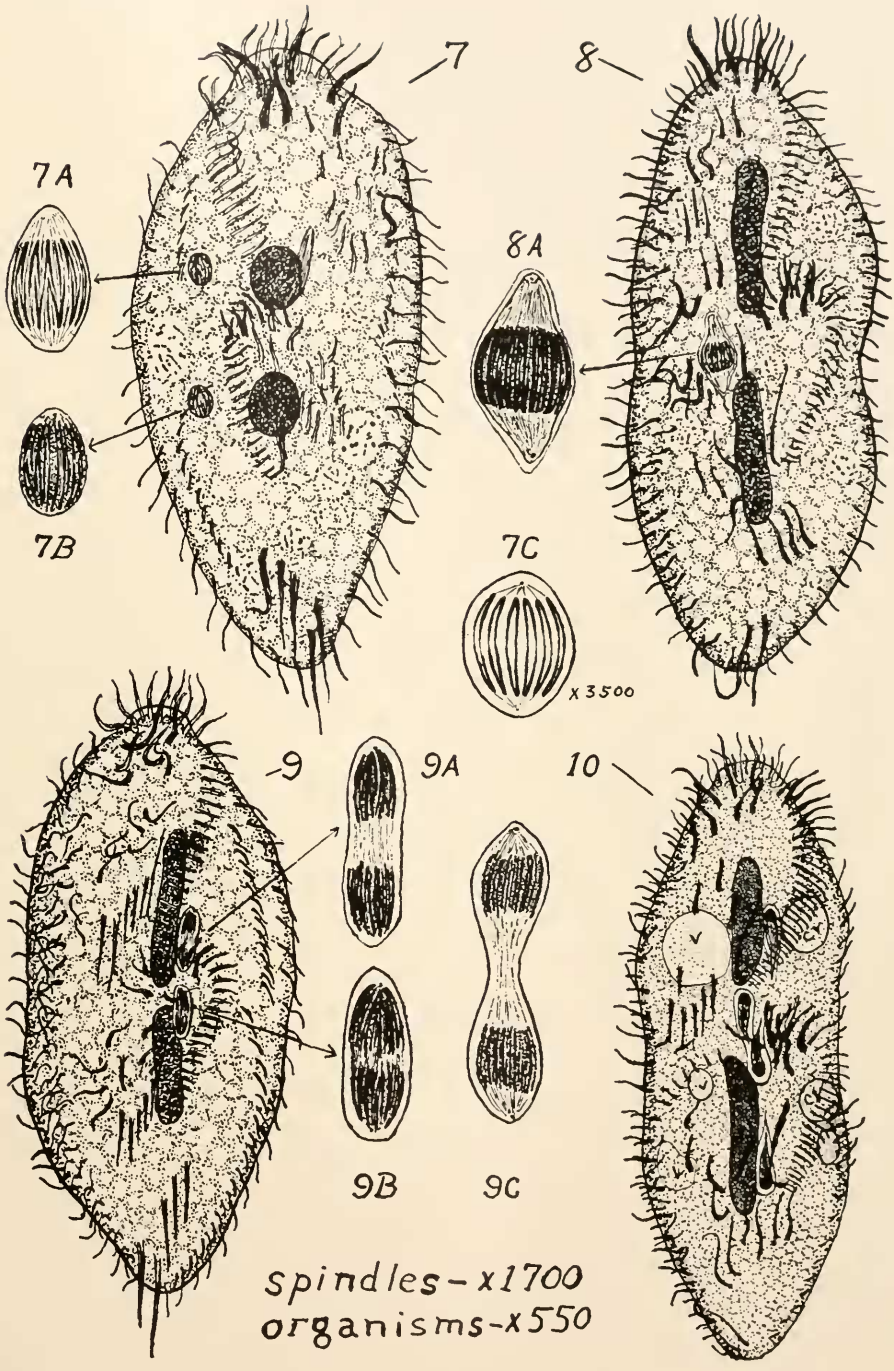
7
8
7A
8A
7B
7C
x 3500
9
9A
10
9B
9C
spindles - x1700
organisms - x550

Plate III.

Final Stages of Division; Cysts.

Fig. 11. The macronuclei are beginning to divide, and some of the old cirri have apparently disappeared. The new ones, originally formed near the adoral zones, are approaching their normal positions.

Fig. 11 *A*. A late stage in the telophase, enlarged from Fig. 11.

Fig. 11 *B*. An earlier telophase.

Fig. 12. The final stage of division.

Fig. 13. An individual which has just divided. The shape differs somewhat from that of a typical vegetative individual, and the anal cirri are not yet quite in their normal positions.

Fig. 14. An early stage of encystment, in which the individual has rounded up, but some of the cirri still remain, and the contractile vacuole is still functional. × 750.

Fig. 15. An encysted animal, in which the cyst wall has just been secreted. × 750.

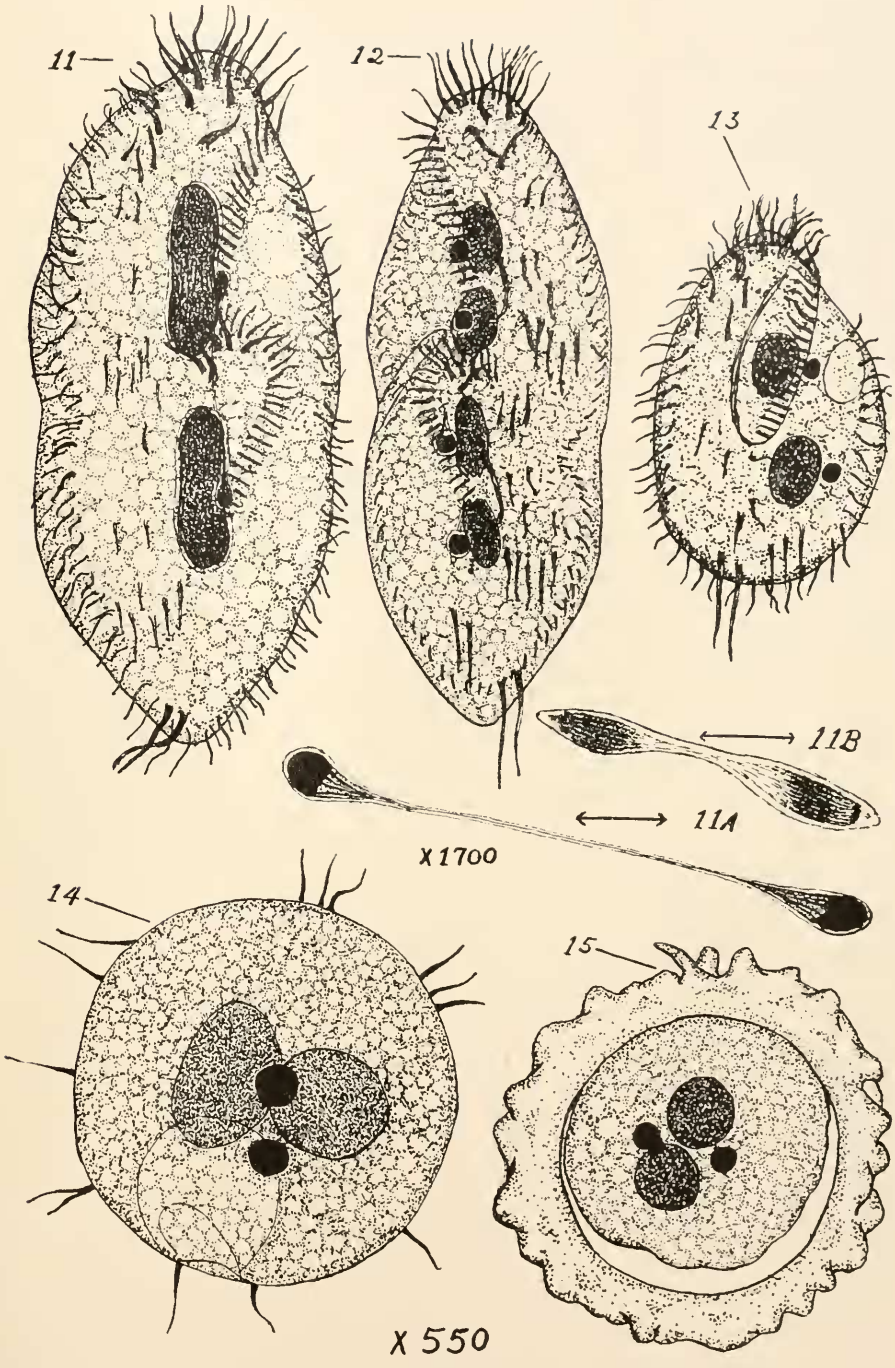

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Plate IV.

Cysts in Various Stages.

Fig. 16. Showing the extrusion of the macronuclei and a small portion of the cytoplasm.

Fig. 17. An encysted animal in which only one micronucleus remains. × 750.

Fig. 18. A cyst containing three nuclei and some darkly staining material which is however not chromatin. × 750.

Fig. 19. A cyst containing seven nuclei, several of which appear to be in mitosis. × 750.

Fig. 20. A cyst in which reorganization is almost complete. × 750.

Fig. 21. Cyst containing a completely reorganized animal. × 750.

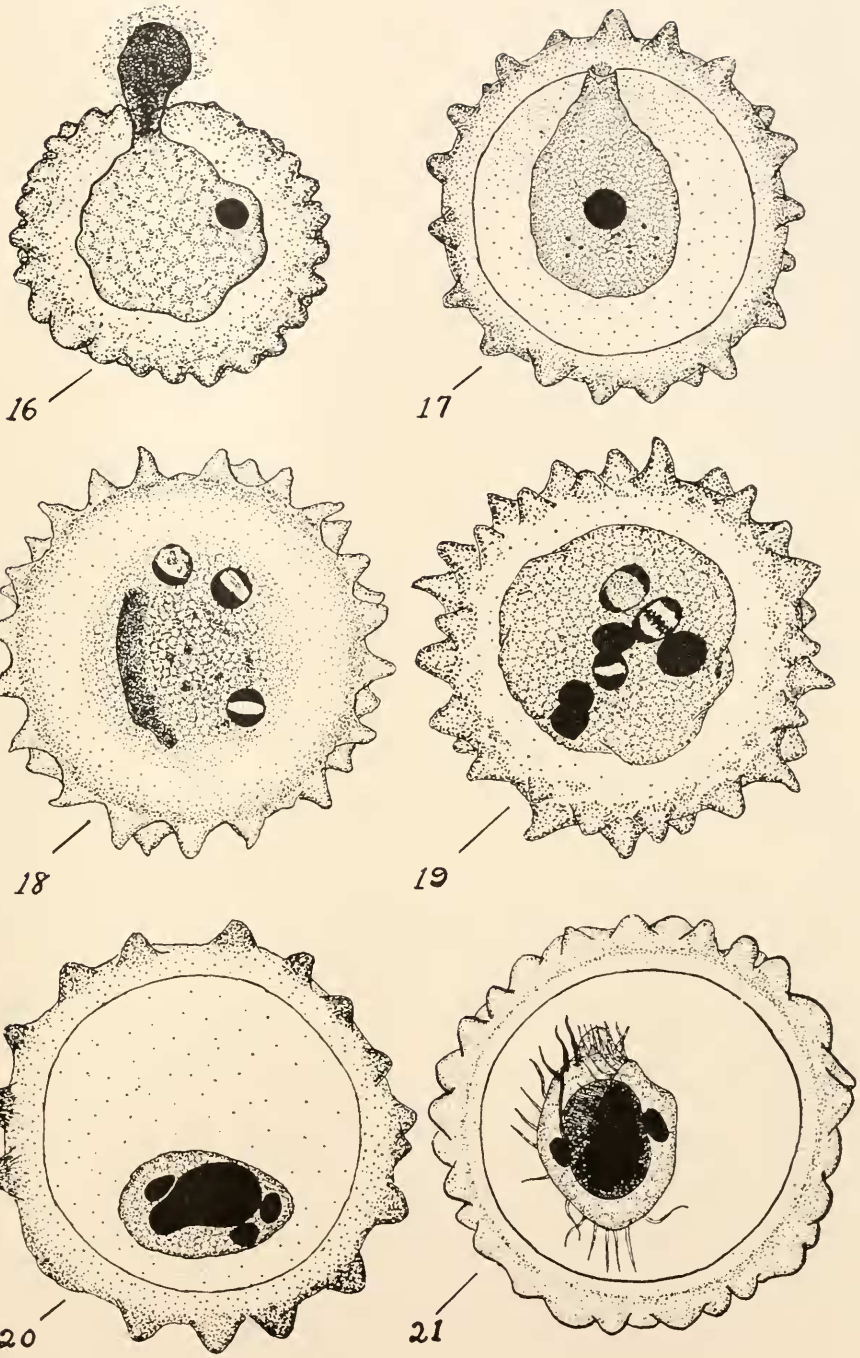

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PLATE V.

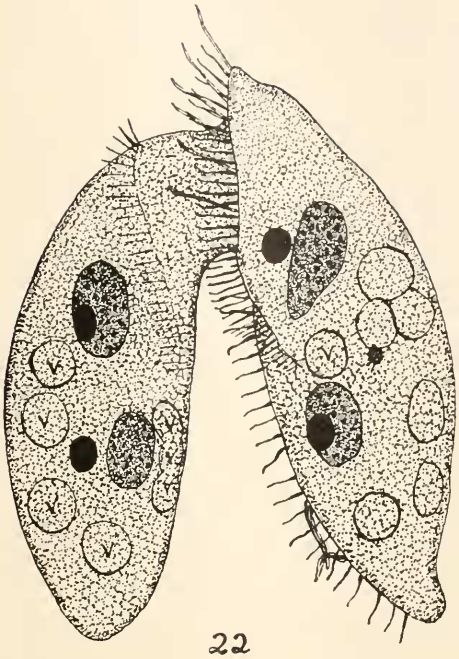
First Maturation Division.

FIG. 22. Initial stage in conjugation, showing the manner of fusion.

FIG. 23. A pair in which the micronuclei have begun to enlarge, preparatory to the first maturation division.

FIG. 24. Here the division centers have made their appearance, and in one nucleus the spindle fibers are becoming visible. Enlarged in 28 *B* and 28 *C*.

FIG. 25. Parachute stage, just prior to the formation of chromosomes. Shown enlarged in Fig. 28 *E*.



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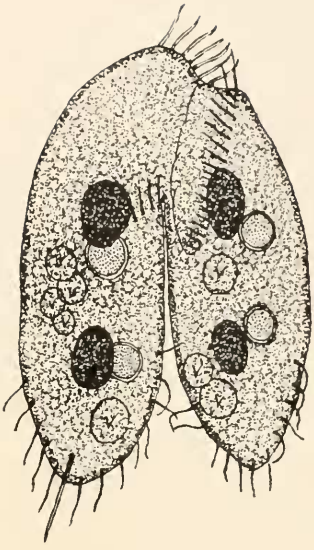

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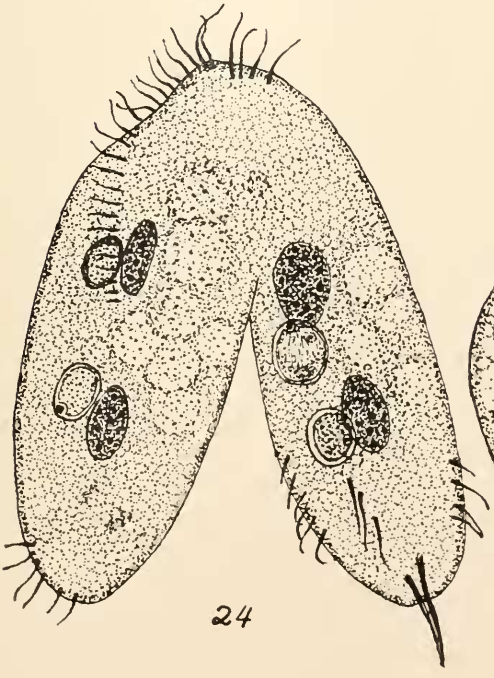

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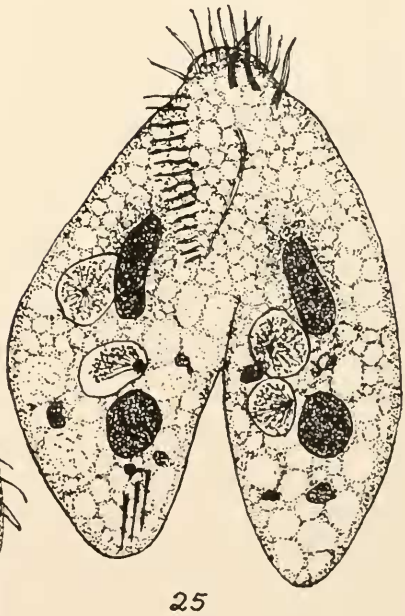

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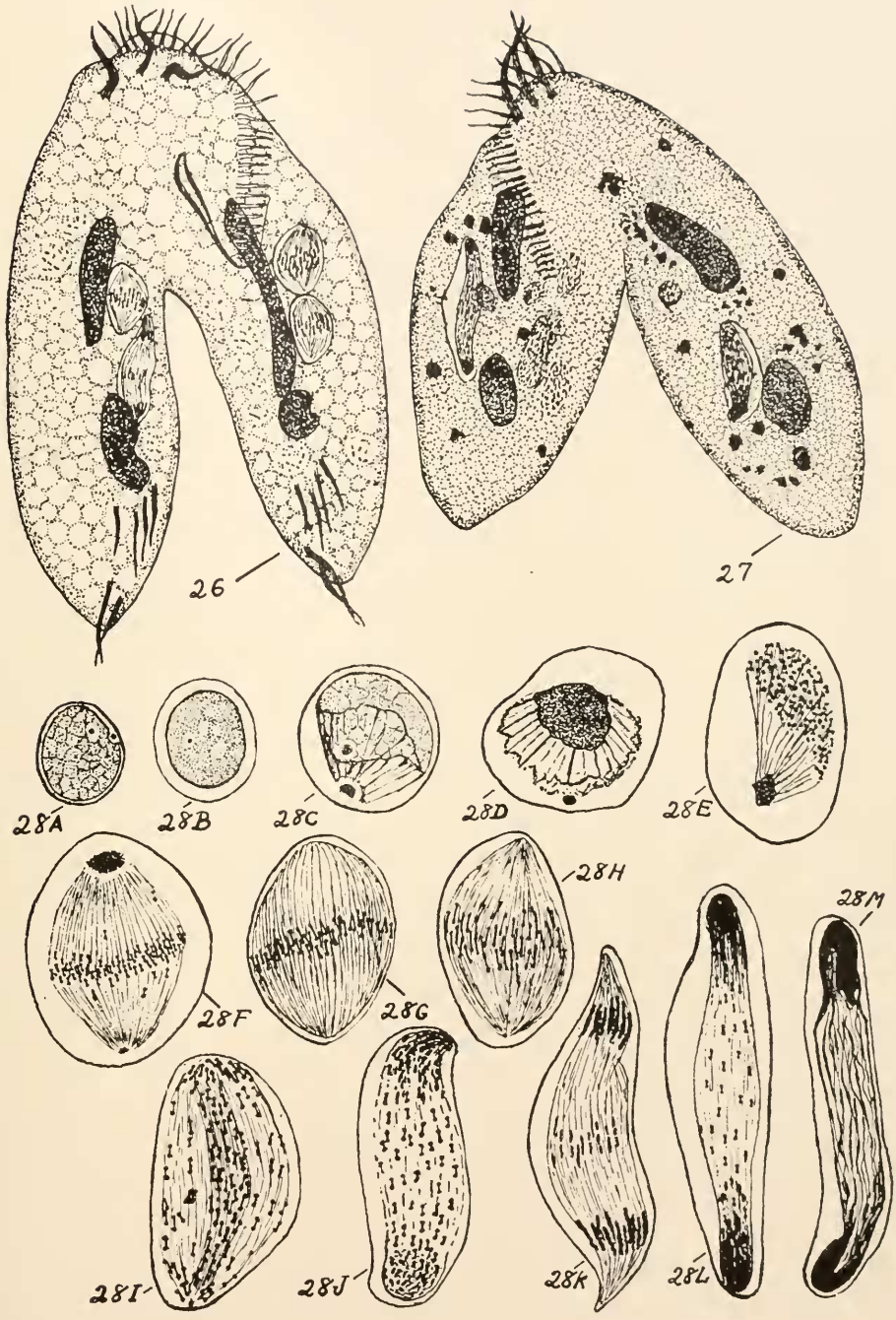
Plate VI.

First Maturation Division.

Fig. 26. Here the micronuclei have entered the anaphase. Two of the spindles are shown enlarged in Figs. 28 *H* and 28 *K*.

Fig. 27. A pair in which a telophase may be seen in each member.

Fig. 28 *A–M*. Various stages of the first maturation division arranged consecutively.



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